

Analysis of the genome sequence of infectious hematopoietic necrosis virus HLJ-09 in China

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Abstract Infectious hematopoietic necrosis virus (IHNV) is a highly contagious disease of juvenile salmonid fish. Six genome target fragments of the complete genome sequence of IHNV HLJ-09 were amplified by RT-PCR, and the 3'-terminal and 5'-terminal region of the genomic RNA were amplified using the RACE method. The complete genome sequence of HLJ-09 comprises 11,132 nucleotides (nt) (Accession number JX649101) and is different from that of other IHNV strains published in GenBank. Homology comparison and phylogenetic analysis of six ORF sequences were carried out using HLJ-09 and other IHNV strains published in GenBank. From phylogenetic tree analysis, the *N* gene, *M* gene, and *P* gene had the closest genetic relationship to IHNV-PRT from Korea. Phylogenetic analysis for the full length of the *G* gene showed that the HLJ-09 strain exhibited very close homology to the ChYa07, RtNag96, RtUi02, and RtGu01 strains from Korea and Japan, indicating that the HLJ-09 strain belonged to the genotype JRt. Ultimately, the Chinese IHNV HLJ-09 strain may have originated in Korea and Japan.

Keywords Infectious hematopoietic necrosis virus (IHNV) · Complete genome · Phylogenetic tree · Sequence analysis

Introduction

IHNV belongs to the genus Novirhabdovirus, family Rhabdoviridae, and produces contagious acute infection in juvenile salmonid fish, resulting in hemorrhage and necrosis of hematopoietic tissue and other internal organs. IHNV is widely prevalent in North America [5], Europe and Asia, and has become a worldwide rainbow trout disease, causing great economic losses in rainbow trout breeding [8]. IHNV is a nonsegmented negative-strand RNA virus with a genome length of approximately 11,100 bp. For the first time, the IHNV genome was sequenced in 1995 [16]. The gene order is 3'-N (nucleocapsid protein)-P (phosphate protein)-M (matrix protein)-G (surface glycoprotein)-NV (non-structural protein)-L (polymerase protein)-5', as in other novirhabdoviruses [22]. Thus far, there is no effective treatment for IHNV. Early vaccination in healthy fish is an effective method to prevent viral infection [6]. Although there are many reports of the IHNV vaccine, only one type of DNA vaccine "APEX-IHN", has acquired vaccine production licenses in Canada [1].

IHNV has only one serotype, and five genotypes have been classified—E, U, M, L, and JRt [14, 19, 20]. In 2003, Kurath conducted a phylogenetic tree analysis for the 303 bp nucleotide hypervariable region of IHNV in the *G* gene and confirmed that the IHNV genotypes of the North American Pacific west coast were U, M, and L [15]. Every genotype has unique territoriality and host specificity. The genotype of IHNV-infected salmon juveniles on

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the Northwest Coast from Oregon to Alaska is U. The genotype of IHNV-infected rainbow trout juveniles in Idaho is M. The genotype of IHNV-infected Chinook salmon juveniles in California is L [7, 18, 23]. IHNV spread from North America to Europe [9] and Japan [24] through rainbow trout roe and juveniles infected with IHNV. The genotypes of IHNV that spread in Europe and Japan are E, U, and M [11]. Phylogenetic tree analysis for the G gene of IHNV isolates in Japan during 1980–1996 indicated that they could belong to genotype JRt, which is derived from the genotype U of North America [17]. In 1991, Korea reported that IHNV infected rainbow trout juveniles for the first time in Kangwon province, and this disease then spread in many areas across Korea. Juveniles were endangered by this disease, but adult fish >300 g were also endangered, a trend that was identical to that of Japan. Kim analyzed the nucleotide sequences of four strains of IHNV and compared them with the different isolates worldwide. The results showed that the isolates from Korea had the closest homology to those from Japan, but not to those from North America or Europe, possibly confirming that IHNV from Korea had been brought from Japan [13].

In this study, we acquired the complete genomic sequence of IHNV HLJ-09 from the isolated IHNV HLJ-09 local strain and then conducted sequence comparative analysis and phylogenetic tree analysis between this strain and other reference strains in GenBank to confirm the genotype of this strain. The data inferred the possible spreading source of this local strain. This study not only

enriched the IHNV biological information database and offered useful information for molecular epidemiological studies of IHNV but also provided a technical platform to study the relationship between the IHNV genome structure and function and to develop a new vaccine.

Materials and methods

Cells and viruses

Endothelial progenitor cells (EPCs) were obtained from American type culture collection (ATCC) and grown in L-15 (Leibovitz) supplemented with 10 % fetal bovine serum (FBS). IHNV HLJ-09 was isolated and purified from dead rainbow trout tissue from an acute disease outbreak at Bohai experimental station, Heilongjiang Academy of Agricultural Sciences in 2009.

Primers design and synthesis

According to IHNV sequences published in GenBank, six pairs of primers were designed and synthesized by comparing higher-homology sequences between IHNV strains. The 3'-terminal and 5'-terminal *Adaptor Primers*, *Outer Primers*, and *Inner Primers* were designed and synthesized according to previous studies [4]. The primer sequences are listed (Table 1).

Table 1 Oligonucleotides used for cloning and sequencing of the IHNV genome

IHNV primers	Sequences	Position (bp)
IHNV 1F	GTATAAGAAAAGTAACTTGAC	1–21
IHNV 1R	CTCCCTCGTATTCATCCTC	2097–2078
IHNV 2F	GCAGGATCCCAAGAGGTGAAG	2033–2053
IHNV 2R	GGAACGAGAGGATTTCTGATCC	3819–3818
IHNV 3F	CAGTGGATACGGACAGATCTC	3767–3787
IHNV 3R	CTGGGAGCTCTCCTGACTTG	5579–5559
IHNV 4F	GTA CTTCACAGATCGAGGATCG	5523–5544
IHNV 4R	CGGGGACTCTGTCTGGAATG	7147–7128
IHNV 5F	CGTACCAGTGAAATACATCGG	7098–7119
IHNV 5R	CAGGTGGTGAAGTAGGTGTAG	9018–8997
IHNV 6F	GAGGGAGTTCTTTGATATTC	8931–8952
IHNV 6R	ATAAAAAAAGTAAACAGAAGGGTTCTC	11,130–11,105
IHNV 3' adaptor primer	GCTGTCAACGATACGCTACGTAACGGCATGACAGTG(T)18	/
IHNV 3' outer primer	CTCCCTCGTATTCATCCTC	/
IHNV 3' inner primer	CGCTACGTAACGGCATGACAGTG	/
IHNV 3'GSP1	TGTTGTTGGGATCTGCGAAAGTGGC	511–487
IHNV 3'GSP2	CTCTGAGTGCCTTGTCATCGTTCCG	193–169
IHNV 5'GSP1	ACTCTCGTCGACACAGTGGTCTCTT	10,479–10,503
IHNV 5'GSP2	ATCTTCAACCTCATCTGCCTCTCT	10,726–10,749

Segmented amplification and splicing of complete genome sequence

The suspension of IHNV HLJ-09 cultured in EPCs was centrifuged at 4 °C, 10,000 r/min for 10 min. Complete RNA was extracted from the above supernatant using the Qiagen RNeasy kit (Qiagen, Valencia, CA).

The RNA was reverse transcribed into cDNA using extracted RNA as the template and Oligo (dT) 15 (TaKaRa) as the primer for RT-PCR. Six target fragments were amplified by PCR using the abovementioned cDNA as a template and six pairs of primers.

In order to identify the 3'-terminal sequence, the cDNA was reverse transcribed into by adding a poly(A) tail to the 3'-terminal of genomic RNA as the template, using the IHNV 3' *Adaptor Primer* as the reverse transcription primer. The outer-nested PCR product was amplified by PCR with this cDNA as the template and IHNV 3' *Outer Primer* and IHNV 3' *GSP1* as primers. The inner-nested PCR product was amplified by PCR with the purified outer-nested PCR product as the template and IHNV 3' *Inner Primer* and IHNV 3' *GSP2* as primers. In order to identify the 5'-terminal sequence, the extracted RNA was modified by the 5' Full RACE Kit (TaKaRa), and then reverse transcribed into cDNA using random primers. The outer-nested PCR product was amplified by PCR using this cDNA as the template and IHNV 5' *Outer Primer* and IHNV 5' *GSP1* as primers. The inner-nested PCR product was amplified by PCR with the purified outer-nested PCR product as the template and IHNV 5' *Inner Primer* and IHNV 5' *GSP2* as primers.

The above PCR products were purified using a DNA Gel Extraction Kit and ligated into a cloning vector (PMD18-T-simple). The ligated products were transformed into competent JM109 cells. For each recombinant strain, at least three positive clones were sequenced by Sangon Biotech in Shanghai.

Sequence analysis of IHNV HLJ-09 cDNA

The complete genome sequences were spliced with six overlapping fragments and the terminal sequences using DNA Star. HLJ-09 genome sequences and other strains of IHNV genome sequences published in GenBank were analyzed at the nucleotide and amino acid levels using DNAMAN and BLAST (NCBI online system). Using MEGA 5.0 and ClustalX, six different protein sequences of IHNV were comparatively analyzed, and the phylogenetic trees were drawn. The strains used, the countries of origin and their accession numbers in GenBank are listed in Table 2.

Results

Identification of the IHNV HLJ-09 complete genome

The positive clones of *F1* had the same sequencing result, similar to *F2–F6*, the 3'-terminal, and 5'-terminal sequences. The complete genome sequences were spliced with six overlapping fragments and the terminal sequences by DNA Star. The complete genome sequence of HLJ-09 comprises 11,132 nucleotides (nt), and the accession number is JX649101.

There are 4 strains of IHNV with a complete genome sequence: WRAC (11,131 bp), X89213 (11,137 bp), 220–90 (11,133 bp), and Ch20101008(11,129 bp). According to NCBI BLAST results, the HLJ-09 strain shared 95.5, 95.9, 95.0, and 99.8 % identity with WRAC, X89213, 220–90, and Ch20101008, respectively (Table 2).

Sequence analysis of IHNV HLJ-09 cDNA

Sequence analysis of the coding regions

Similar to other novirhabdoviruses, the IHNV HLJ-09 genome encodes six proteins; N (nucleocapsid protein), P (phosphate protein), M (matrix protein), G (surface glycoprotein), NV (non-structural protein), and L (polymerase protein). The order is 3'-N-P-M-G-NV-L-5'. Each gene is separated by a noncoding region. The lengths of their ORFs and predicted coding amino acids are outlines in Table 3.

The ORF of the *N* gene (ORF 1) is located at 175–1350 bp in the cDNA and encodes 391 amino acids with a molecular weight of 42.0 kDa. Transcription of the *N* gene starts at the conservative sequence “CGUG” and polyadenylation signal. The length of the 5' untranslated region is 112 bp (from the translation initiating signal to “AUG”), and the length of the 3' untranslated region is 80 bp (from the termination codon to the transcription termination signal). Online prediction showed that the *N* protein included 9 serines, 6 threonines, and 3 tyrosines as potential phosphorylation sites. The *N* gene of HLJ-09 shares high homology with that of Carson-89, LWS-87, Round Butte 1, RB-76, HV7601, and Ch20101008 (95.6–100.0 %) (Table 2).

The length of the *P* gene (ORF 2) is located at 1466–2158 bp in the cDNA and encodes 230 amino acids with a molecular weight of 26.0 kDa. The length of the 5' untranslated region is 33 bp, and the length of the 3' untranslated region is 41 bp. Online prediction showed that the *P* protein included 5 serines, 4 threonines, and 1 tyrosine as potential phosphorylation sites. The *P* gene of HLJ-09 shares high homology with that of strain K,

Table 2 Percent (%) nucleotide identity of the IHNV strain HLJ-09 with other IHNV strains

Strain	Accession no.	Country	3'	N	P	M	G	NV	L	5'	Complete genome
AU-9695338	FJ265712	Australia	/	93.8	/	/	/	/	/	/	/
DE-DF 13/98 11621	FJ265714	Germany	/	93.8	/	/	/	/	/	/	/
IT-217A	FJ265710	Italy	/	94.0	/	/	/	/	/	/	/
LR-80	AY442514/L40878	USA	/	93.9	/	/	94.6	95.8	/	/	/
SRCV	AY442517/L40881	USA	/	93.5	/	/	94.4	/	/	/	/
Carson-89	AY442508	USA	/	95.6	/	/	/	/	/	/	/
LWS-87	AY442515	USA	/	95.7	/	/	/	/	/	/	/
LB91KI	AY438975	USA	/	95.3	/	/	/	/	/	/	/
RB-76	AY442516/L40880/U15170	USA	/	95.8	/	/	95.3	97.3	/	/	/
Round Butte 1	U50402/U50401/U47846	USA	/	95.7	/	/	95.5	97.0	/	/	/
RU-FR-1	FJ265715	Russia	/	95.2	/	/	/	/	/	/	/
HV7601	AB231684/B231658/AB231659	Japan	/	96.4	95.5	98.3	/	97.3	98.3	/	/
Baker Lake 94	HM236352/HQ189136	USA	/	/	94.9	98.3	/	/	/	/	/
HLM-3	AB288093	Japan	/	/	/	/	/	/	/	/	/
RB1	HM236353	USA	/	/	/	98.3	/	/	/	/	/
Hni00	AB288099	Japan	/	/	/	97.8	/	/	/	/	/
Hen00	AB288100	Japan	/	/	/	98.1	/	/	/	/	/
CH-118-02	EU676197	Switzerland	/	/	/	/	94.6	/	/	/	/
F28701	EU331446	France	/	/	/	/	94.4	/	/	/	/
CH-247-00	EU676198	Switzerland	/	/	/	/	94.2	/	/	/	/
CH-308-97	EU676199	Switzerland	/	/	/	/	94.5	/	/	/	/
IIO	FJ711518	Italy	/	/	/	/	94.6	/	/	/	/
Dau832-94	EU676209	Germany	/	/	/	/	94.8	/	/	/	/
Dfr100-96	EU676217	Germany	/	/	/	/	94.8	/	/	/	/
Dau819-96	EU676208	Germany	/	/	/	/	94.9	/	/	/	/
I166-05	FJ711510	Italy	/	/	/	/	94.4	/	/	/	/
Fp7946	EU331455	France	/	/	/	/	94.7	/	/	/	/
Fo43725	EU331452	France	/	/	/	/	94.6	/	/	/	/
Fn150974	EU33451	France	/	/	/	/	94.7	/	/	/	/
CST-82	L40875	USA	/	/	/	/	94.6	96.7	/	/	/
FF030-91	DQ164103	USA	/	/	/	/	94.3	/	/	/	/
HO-7	L40876	USA	/	/	/	/	94.4	95.2	/	/	/
Col-80	L40873	USA	/	/	/	/	94.2	/	/	/	/
Col-85	L40874	USA	/	/	/	/	94.2	95.2	/	/	/
BLk94	DQ164100	USA	/	/	/	/	95.4	/	/	/	/
Auke77	DQ164099	USA	/	/	/	/	95.9	/	/	/	/
RtToya80	AB250935	Japan	/	/	/	/	96.5	/	/	/	/
RtWanJu09	HM021723	Korea	/	/	/	/	94.8	/	/	/	/
ChYa07	FJ230851	Korea	/	/	/	/	97.2	/	/	/	/
RtNag96	AB250932	Japan	/	/	/	/	97.6	/	/	/	/
RtGu01	AB288206	Korea	/	/	/	/	97.9	/	/	/	/
RtUi02	AB288207	Korea	/	/	/	/	97.4	/	/	/	/
fs8	AY780893	Germany	/	/	/	/	/	96.1	/	/	/
Cro/05	EU219617	Croatia	/	/	/	/	/	95.8	/	/	/
NoviTun-1	DQ462312.1	North Africa	/	/	/	/	/	97.3	/	/	/

Table 2 continued

Strain	Accession no.	Country	3'	N	P	M	G	NV	L	5'	Complete genome
fs832	AY780894	Germany	/	/	/	/	/	97.0	/	/	/
fs42	AY780896	Germany	/	/	/	/	/	96.7	/	/	/
fsvi100	AY780898	Germany	/	/	/	/	/	96.4	/	/	/
fs62	AY780897	Germany	/	/	/	/	/	97.0	/	/	/
IHNV -PRT	AY673683/AY673686/AY673685/ AY673684/AY673687	Korea	/	93.9	95.5	97.8	95.4	95.2	/	/	/
Strain K	X73872	USA	/	93.5	94.4	97.4	94.8	97.0	97.3	/	/
WRAC	L40883	USA	.95.0	93.6	93.9	97.4	94.4	95.8	96.6	87.3	95.5
X89213	X89213	USA	96.7	93.7	94.4	97.4	94.8	97.0	97.1	86.3	95.9
220-90	GQ413939	USA	93.3	93.4	93.2	97.6	93.9	95.5	96.0	87.2	95.0
Ch20101008	KJ421216	China	98.3	100.0	99.9	99.9	100.0	97.0	99.8	96.2	99.8

The percentage identities of the IHNV strain HLJ-09 with other IHNV strains were computed using DNAMAN. All of the above gene sequences are available in GenBank. Only nucleotide sequences were used for analysis. These percent values are rounded to one decimal place

Table 3 Genomic features and structural gene products of the IHNV HLJ-09 strain

Gene	Start (bp)	End (bp)	5'UTR (bp)	ORF length (bp)	3'UTR (bp)	Total length ^a (bp)	Protein size (aa)	MW ^b (kDa)
Leader	1	60	/	/	/	60	/	/
N	63	1430	112	1176	80	1638	391	42.0
P	1433	2199	33	693	41	767	230	26.0
M	2202	2945	53	588	103	744	195	22.0
G	2948	4567	51	1524	42	1620	508	56.6
NV	4570	4938	26	336	7	369	111	13.2
L	4941	11031	76	5961	54	6091	1986	225.0
Trailer	11,032	11,132	/	/	/	101	/	/

^a Total length of a gene including 5'UTR, ORF, and 3'UTR

^b Predicted molecular weight of proteins are rounded to one decimal place

X89213, Baker Lake 94, HV7601, IHNV-PRT, and Ch20101008 (94.4–99.9 %) (Table 2).

The length of the *M* gene (ORF 3) is located at 2255–2842 bp in the cDNA and encodes 195 amino acids with a molecular weight of 22.0 kDa. The length of the 5' untranslated region is 53 bp, and the length of the 3' untranslated region is 103 bp. The *M* gene of HLJ-09 shares high homology with that of IHNV-PRT, Hni00, Hen00, HV7601, Baker Lake 94, and Ch20101008 (97.8–99.9 %) (Table 2).

The ORF of the *G* gene (ORF 4) is located at 2999–4525 bp in the cDNA, and the gene encodes 508 amino acids with a molecular weight of 56.6 kDa. The length of the 5' untranslated region is 51 bp, and the length of the 3' untranslated region is 42 bp. Online prediction showed that the *G* protein included 17 serines, 6 threonines, and 6 tyrosines as potential phosphorylation sites, and 56–59 aa (NASQ), 379–382 aa (NFTK), 401–404 aa (NTTI), and 438–441 aa (NETE) as assumed *N*-glycosylation sites.

Except for 379–382 aa (NFTK), the remaining 3 sites were identical to that of IHNV 220-90 [3]. By comparative analysis of the *G* protein sequences of 37 strains published in GenBank, the regions of 32–52 aa, 131–204 aa, 287–369 aa, and 380–416 aa were highly conserved regions, while the regions of 247–257 aa and 269–276 aa were hypervariable regions. Online prediction showed that the *G* protein could be composed of an N-terminal signal peptide (1–20 aa), an outer functional area (21–459 aa), a transmembrane region (460–482 aa), and an inner functional area (483–508 aa). When the *G* genes were compared, the HLJ-09 strain shared 96.5–100.0 % identity with RtToya80, ChYa07, RtUi02, RtNag96, RtGu01, and Ch20101008 strains (Table 2), which were identical with *G* proteins. Among them, the *G* gene of HLJ-09 shared as high as 97.9 % identity with that of the RtGu01 strain from Korea regarding both nucleotide and protein.

The ORF of the *NV* gene (ORF 5) is located at 4596–4931 bp in the cDNA and encodes 111 amino acids

with a molecular weight of 13.2 kDa. The length of the 5' untranslated region is 26 bp, and the length of the 3' untranslated region is 7 bp. Online prediction showed that NV protein included 1 serine, 1 threonine, and 2 tyrosines as potential phosphorylation sites. The function of the NV gene is unclear. NV protein is a non-structural protein, related to the process of viral infection.

The ORF of the *L* gene (ORF 6) is located at 5017–10,977 bp in the cDNA, and the gene encodes 1986 amino acids with a molecular weight of 225.0 kDa. The length of the 5' untranslated region is 76 bp, and the length of the 3' untranslated region is 54 bp. Online prediction showed that the *L* protein included 72 serines, 42 threonines, and 11 tyrosines as potential phosphorylation sites. The *L* gene of HLJ-09 shares high homology with that of 220-90, WRAC, X89213, strain K, HV7601, and Ch20101008 (96.0–99.8 %) (Table 2). The *L* protein of HLJ-09 shares approximately 60, 85, and 59 % homology with that of viral hemorrhagic septicemia virus (VHSV), hiram rhabdovirus (HIRRV), and snakehead rhabdovirus (SHRV), respectively.

Analysis of genomic terminal sequences

The length of the 3' leader untranslated region sequence is 60 bp, and the length of the 5' trailer untranslated region sequence is 101 bp. The 3'-terminal sequence of the HLJ-09 genomic RNA shares high homology with WRAC, X89213, 220-90, and Ch20101008 (approximately 95.0, 96.7, 93.3, and 98.3 %, respectively). However, the 5'-terminal sequence of the HLJ-09 genomic RNA shares less homology with the above strains (approximately 87.3, 86.3, 87.2, and 96.2 %, respectively).

Phylogenetic tree analysis

Homology comparison and phylogenetic tree analysis for the six ORF sequences were carried out between HLJ-09 and other IHNV strains published in GenBank using MEGA 5.0 and ClustalX [12]. From phylogenetic tree analysis, except for Ch20101008 from China, the *N* gene, *P* gene of HLJ-09 had the closest genetic relationship to IHNV-PRT from Korea. The *M* gene of HLJ-09 had the closest genetic relationship to Hen00, Hni00 from Japan and IHNV-PRT from Korea. The *NV* gene of HLJ-09 had the closest genetic relationship to CST-82 and WRAC. The *L* gene of HLJ-09 had the closest genetic relationship to HV7601 from Japan (Fig. 1). Phylogenetic analysis of the *G* gene showed that the HLJ-09 strain exhibited very close homology to the ChYa07, RtNag96, RtUi02, and RtGu01 strains from Korea and Japan, and indicated that the 37 IHNV strains belong to different genogroups (Fig. 2).

Discussion

Complete genomic sequence acquisition and bioinformatics analysis are the premises to study viral structure and function. Except for HLJ-09, there are only four IHNV strains for which the complete genome sequences are published in GenBank—WRAC, X89213, 220-90, and Ch2010100—the information from which is not sufficient to understand the genetic evolution of IHNV. It is important to gain more exact genomic sequences and information regarding new strains to investigate the molecular epidemiology of IHNV.

In this study, we cloned all the gene segments of IHNV HLJ-09 according to the traditional method of genome cloning and then joined them for the complete genomic sequence. Six gene fragments were amplified from viral cell culture supernatants by RT-PCR. High-fidelity M-MLV reverse transcriptase and Primer STAR.HS DNA polymerase were used for sequence authenticity and maximum prevention of viral sequence distortion caused by mismatch. Reducing the number of PCR cycles decreased the rate of base mismatch. Each fragment sequence was determined after being repetitively sequenced at least three times. Viral genomic terminal sequences were difficult to obtain, so the RACE method was used in this study. The 5'-terminal sequence of the IHNV genome was acquired using the 5' Full RACE Kit. However, the 3'-terminal sequence of the IHNV genome is complex, most likely due to the IHNV 3'-terminal region not containing a poly (A) tail. Thus, at first, the poly(A) tail sequence was added to the 3'-terminal region of the genomic RNA by poly(A) polymerase according to Ammayappan [4], and the 3'-terminal sequence of IHNV was then acquired by RACE.

Like other Rhabdoviruses, the bases of the 3' leader (1–16 bp) and 5' trailer (24–36 bp) of HLJ-09 show complete complementarity and could form a stable structure. This feature is critical to viral replication. Due to the 5'-terminal sequence of the IHNV HLJ-09 strain having 12 different bases from the other four strains in 101 bp, the homology is lower. However, these mutations were not located in the complementary sequences of the 3' Leader and 5' Trailer. By comparison, the 5'-terminal sequence of HLJ-09 genomic RNA ends of “U” and “G” is identical to which of WRAC, whereas the 220-90 strain has one more “U”, then the X89213 strain and Ch20101008 lack “U” or “G”.

The sequence of the untranslated region in ITS includes a transcription termination signal “UCURUCU⁷”, a poly(A) tail formed by a multiple U repeat, a dinucleotide sequence “AC” or “GC” (untranslated) and a transcription start signal “CGUG”, which is in complete agreement with

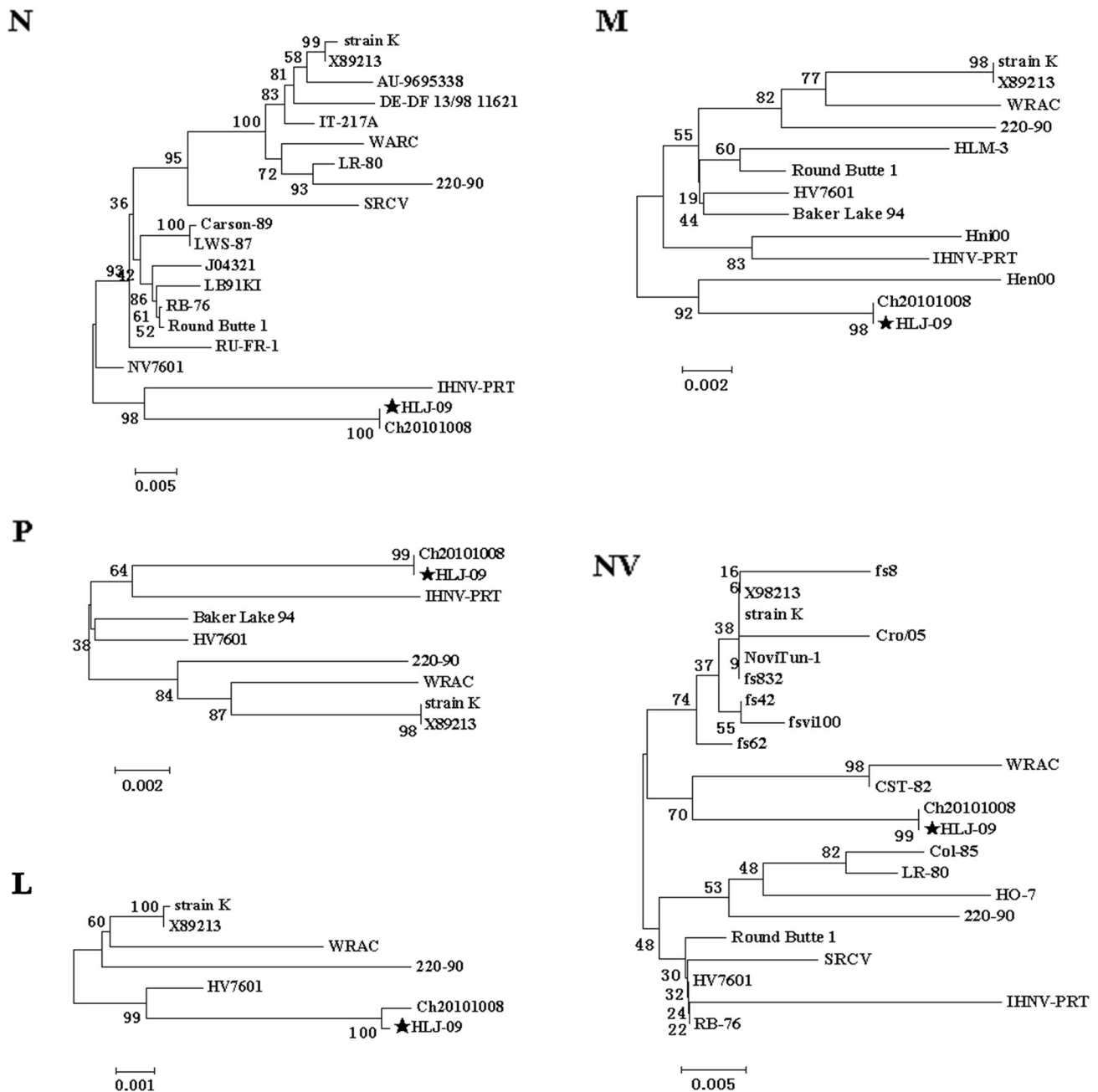


Fig. 1 Phylogenetic tree analysis of sequences of nucleocapsid (N), matrix (M), phosphoprotein (P), non-virion protein (NV), and polymerase protein (L) of various IHNV strains. Information about the IHNV strains used in this analysis is described in Table 1. IHNV HLJ-09 strain

is marked with five-pointed star. Phylogenetic tree analysis was conducted by neighbor-joining method using 1000 bootstrap replications. The scale at the bottom indicates the number of substitution events and bootstrap confidence values are shown at branch nodes

the feature of the internally transcribed spacer of nonvirhabdoviruses.

In 2003, Kurath had analyzed 303 nucleotides in hyper-variable region of G gene and identified the genotypes of IHNV [15]. Five genotypes of IHNV have been identified—E, M, L, U, and JRt. Phylogenetic analysis of the complete sequence of the G gene showed that the HLJ-09 strain

exhibited very close homology to the ChYa07, RtNag96, RtUi02, RtGu01 strains from Korea and Japan, indicating that the HLJ-09 strain belongs to the genotype JRt and that HLJ-09 may be derived from Korea or Japan. The comparison of G protein sequences in different strains revealed that 11 amino acids of the G protein were peculiar to the genotype JRt, including 75S, 84V, 91A, 130R, 215A, 217P, 220N,

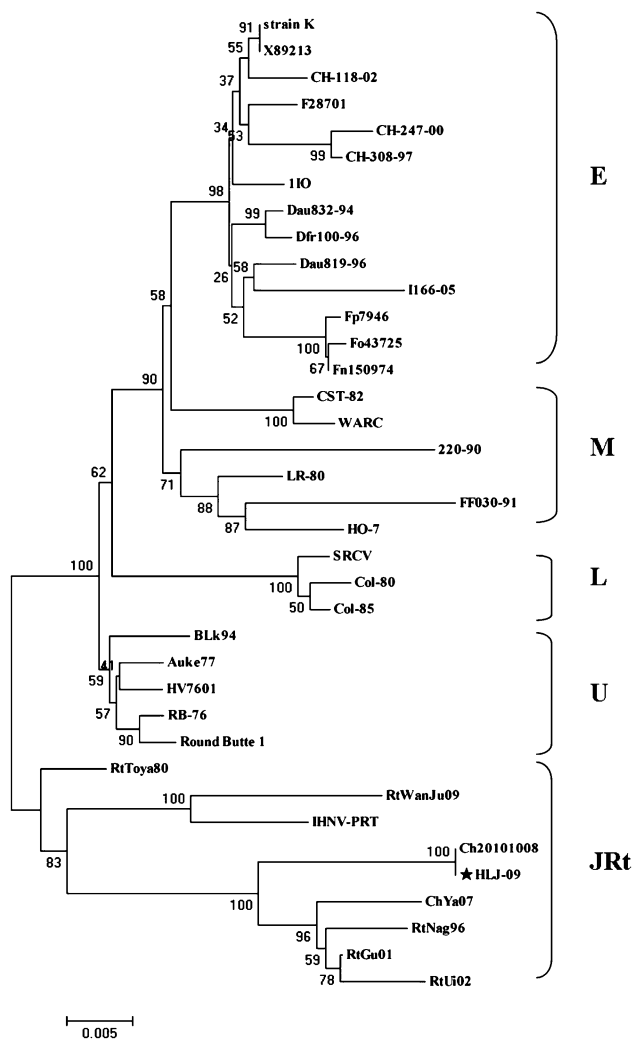


Fig. 2 Phylogenetic tree analysis of glycoprotein (G) sequences of various IHNV strains. *Brackets* indicate the five genogroups, E, M, L, U, and JRt. IHNV HLJ-09 (five-pointed star) is grouped under JRt genogroup. Information about the IHNV strains used in this analysis is described in Table 1. Phylogenetic tree analysis was conducted by neighbor-joining method using 1000 bootstrap replications. The *scale* at the *bottom* indicates the number of substitution events and bootstrap confidence values are shown at branch nodes

232A, 376H, 379N, and 402I, reflecting the evolutionary characteristics of the genotype JRt [2, 10, 21]. Among them, 205I, 230S, 286E, and 441E are peculiar to HLJ-09.

Conclusions

In this study, we acquired the complete genomic sequence of IHNV HLJ-09 from the separated IHNV HLJ-09 local strain, studied the basic molecular biological characteristics of IHNV HLJ-09, confirmed the genotype and inferred the possible spreading source of IHNV HLJ-09 for the first time, all of which enriched the IHNV biological information database and offered useful information for molecular

epidemiological studies of IHNV. Thus, this study provided a technical platform for studying the relationship of IHNV genome structure and function to develop a new vaccine.

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Author contributions C. Wang performed the sequence analysis of IHNV HLJ-09 cDNA and wrote the manuscript. L. L. Zhao performed the complete genome PCR amplification and cloning of IHNV HLJ-09 cDNA. Y. J. Li and M. Liu pointed this subject in the right direction and modified the manuscript. L. J. Tang, X. Y. Qiao, and Y. P. Jiang provide technical information.

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